PROCEDURES

A. TISSUES SAMPLED FOR ANTIBACTERIAL ACTIVITY

For the great majority of studies, we used hemolymph. We primarily tested hemolymph because it was easily and reproducibly collected for quantitative studies and may act as a reservoir for antimicrobial agents, which may then be transported into the carapace by epithelial cells or tegumental glands.

Blue crabs were collected from various sites in the Albemarle-Pamlico Estuary by commercial fishermen and N.C. Division of Marine Fisheries personnel (Figures 1,2). Crabs were sampled immediately on site by severing the 5th pereiopod at the merepodite, collecting the hemolymph in a sterile tube and allowing it to clot on ice. Samples were transported to the laboratory, homogenized to break up the clot and centrifuged at 50,000 x g for 20 min. The resulting supernatant was frozen at -70^{o}C for later testing for antimicrobial activity as described in part D (p. 21), or stored at 4^{o}C for analysis of hemocyanin content as described in part H (p. 24).

The entire dorsal cuticle (epicuticle, procuticle, and epidermis) of 6 soft crabs was harvested and pooled as one group. Soft crabs had been purchased from a commercial shedding operation near Beaufort and maintained in flowing seawater at the National Marine Fisheries Service Laboratory, Beaufort, for several days prior to sampling. Biochemical extraction was performed as described below (part F.4).

Digestive gland was also collected from individual blue crabs and frozen at -70^{o}C for later examination for metal content.

B. BACTERIAL CULTURES

Cultures were taken from a) clinically normal crabs collected from Core Sound, b) shell disease lesions of crabs from the Pamlico River and c) clinically normal carapace of group b) crabs. Samples a) and c) were taken from the right anterior quadrant of the dorsal carapace. Sites were sampled by scraping the carapace with a sterile plastic loop and applying the loop to a small area of a culture plate having trypticase soy agar with 5% defibrinated sheep blood. The inoculum was then spread on the plate using a sterile swab. Plates were incubated at room temperature and predominant colonies were picked after 24 hr incubation. Isolates were purified by restreaking three times and then identified using standard procedures (Krieg 1986).